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OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

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MEMORANDUM

Date: November 25, 2009

SUBJECT: Fluopyram: Report of the Cancer Assessment Review Committee

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CAS No.: 658066-35-4
40 CFR: N/A

FROM: Jessica Kidwell, Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509P)

THROUGH: Mary Manibusan, Co-chair
Cancer Assessment Review Committee
Health Effects Division (7509P)

TO: Myron Ottley, Toxicologist
Paula Deschamp, Branch Chief
Registration Action Branch III
Health Effects Division (7509P)

Cynthia Giles-Parker
Fungicide Branch
Registration Division (7505P)

The Cancer Assessment Review Committee met on July 8, 2009 to evaluate the cancer classification of Fluopyram in accordance with the *EPA's Final Guidelines for Carcinogen Risk Assessment* (March, 2005). Attached please find the final Cancer Assessment Document.

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF

FLUOPYRAM

PC CODE 080302

November 25, 2009

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

FLUOPYRAM CANCER ASSESSMENT DOCUMENT FINAL

DATA PRESENTATION: _____
Myron Ottley, Toxicologist

DOCUMENT PREPARATION: _____
Jessica Kidwell, Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the assessment unless otherwise noted.)

Gregory Akerman _____

Lori Brunsman, Statistician _____

Marion Copley _____

Kit Farwell _____

Ray Kent _____

Mary Manibusan, Co-Chair _____

Karlyn Middleton on leave

Rob Mitkus _____

Esther Rinde _____

NON-COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the pathology report)

John Pletcher, Consulting Pathologist _____

OTHER ATTENDEES: Leung Cheng, Barry O'Keefe, Joel Patterson (PMRA observer), Meheret Negussie, Steve Funk, Whang Phang, Doug Wolf, Sheila Healy, Paula Deschamp, Michael Khan; On conference phone with Global Review Partners- Germany (Philip Marx-Stölting, David Schumacher) and Canada (Catherine Adcock, Michael Honeyman, Martin Gerrits, Carmen Cheung)

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EXECUTIVE SUMMARY

On July 8, 2009, the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of fluopyram. This is the first assessment of fluopyram by the CARC. Fluopyram is a global review chemical in partnership with Canada and Germany. Germany has the work share lead for review of toxicology data.

Myron Ottley of Risk Assessment Branch III presented the chronic toxicity/carcinogenicity study in Wistar rats and the carcinogenicity study in C57BL/C6 mice. In a combined chronic toxicity and carcinogenicity study, groups of 60 male and female Wistar rats were fed a diet containing 0, 30, 150 and 750 ppm (males) and 0, 30, 150 and 1500 ppm (females) fluopyram for 24 months. In males, the top dose level of 750 ppm had to be reduced to 375 ppm from week 85 onwards because of the high mortality in this group. Over the whole study period, these dietary concentrations corresponded to a mean daily intake of 0, 1.20, 6.0 and 29 mg/kg bw in male rats or 1.68, 8.6 and 89 mg/kg bw in females. In addition, satellite groups of 10 animals per sex and dose were subject to interim sacrifice after 1 year. Groups of 60 male and female C57BL/6J mice were fed diets containing 0, 30, 150 or 750 ppm of fluopyram (corresponding to a mean compound intake of 0, 4.2, 20.9 and 105 mg/kg bw/day in males and 0, 5.3, 26.8 and 129 mg/kg bw/day in females, respectively) for up to 78 weeks. After 52 weeks, 10 males and 10 females from each group which had been allocated to the chronic phase of the study were killed and necropsied. The remaining 50 animals per sex and group (allocated to the carcinogenicity phase) continued treatment until scheduled sacrifice after at least 78 weeks of treatment. Information on mutagenicity, structure activity relationship and mode of action data for thyroid and liver tumors was also presented.

The CARC concluded the following:

Carcinogenicity

Rat

· Administration of fluopyram resulted in the induction of liver tumors in female Wistar rats. There were statistically significant trends for liver adenomas ($p<0.01$), carcinomas ($p<0.05$) and combined liver adenomas and carcinomas ($p<0.01$). There were significant pair-wise comparisons of the 1500 ppm dose group with the controls for liver adenomas at $p<0.05$ and for combined liver adenomas and carcinomas at $p<0.01$. When compared to historical control data (uncensored data) from the testing laboratory, the incidence of hepatocellular adenomas in the female high dose group (9/55, 16%) was outside the range of the historical control group (range, 0 - 5%; average, 1.9%). Similarly, the incidence of hepatocellular carcinomas in the female mid (2/56, 4%) and high dose groups (3/55, 5%), while not statistically significant by pair-wise comparison, exceeded the range of the historical control group (no carcinomas observed in 10

studies from 2000 - 2006) and was considered to be biologically relevant. There were no statistically significant trends or significant pair-wise comparisons of the dosed groups with the controls for the male rats. **The CARC considered the liver tumors in female Wistar rats to be treatment-related.**

· *Adequacy of Dosing*: The doses tested were considered to be adequate and not excessive to assess carcinogenic potential in both sexes. **In female rats**, the highest dose tested of 1500 ppm was considered to be adequate. This was based on a 20% decrease in mean body weights and increased liver weights (mean: +39%, absolute: +56%), which were dose-related and correlated well with findings such as prominent lobulation and hypertrophy. Other effects that occurred at the high dose included thyroid follicular cell hypertrophy and colloid alteration, chronic progressive nephropathy and kidney tubular dilatation, and retinal atrophy and lenticular degeneration of the eye. **In male rats**, the top dose level of 750 ppm, an excessive dose, had to be reduced to 375 ppm from week 85 onwards because of the high mortality in this group. At the termination of the study, mortality among high-dose males was 50% of control values. The dose of 375 ppm, which is roughly one-half the excessive dose of 750 ppm, however, was considered to be adequate. This was based on decreased mean body weight, increased liver weight, liver hypertrophy, follicular cell hypertrophy and colloid alteration, chronic progressive nephropathy and tubular dilatation and tubular hypertrophy (males) all observed at the high dose.

Mouse

· Administration of fluopyram resulted in the induction of thyroid follicular cell tumors in male C57BL/6J mice. Male mice had a statistically significant trend at $p<0.01$ and a significant pair-wise comparison of the 750 ppm dose group with the controls at $p<0.05$ for thyroid follicular cell adenomas. There were no statistically significant trends or significant pair-wise comparisons of the dosed groups with the controls for the female mice. When compared to historical control data (uncensored data) from the testing laboratory, the incidence of thyroid follicular cell adenomas in the male high dose group (7/48, 15%) was outside the range of the historical control group (range, 0 - 2%; average, 0.4%). **The CARC considered the thyroid follicular cell adenomas in male C57BL/6J mice to be treatment-related.**

· *Adequacy of Dosing*: The high dose of 750 ppm was considered to be adequate, and not excessive, to assess carcinogenic potential in both male and female mice, based on the following: 1) Increased absolute and relative liver weights in males and females at the high- and mid-dose levels. These changes were dose-related, and correlated well with macroscopic findings (dark/enlarged livers) and non-neoplastic histopathological lesions (eosinophilic foci [females], hepatocellular hypertrophy [both sexes], cholestasis [males], single cell degeneration/necrosis [males]); 2) Increased incidence of thyroid follicular cell hyperplasia in both sexes at the high dose; and 3) Decreased absolute and relative kidney weights in both sexes at the high dose level, along with increased incidence and/or severity of kidney lesions (bilateral cortical basophilic tubules, hyaline casts and interstitial mononuclear cell infiltrates, glomerular congestion/

hemorrhage, and amyloid deposition) in females.

Mutagenicity

There is no concern for mutagenicity.

Structure-Activity Relationship (SAR)

The SAR data do not inform the mode of action.

Mode of Action

The CARC concluded that insufficient data were provided to support definitive modes of action for the induction of liver tumors in female rats or thyroid follicular cell tumors in male mice. The main deficiency included a lack of dose-response concordance with key events and tumors.

Classification and Quantification of Carcinogenic Potential

In accordance with the EPA's *Final Guidelines for Carcinogen Risk Assessment* (March, 2005), the CARC classified fluopyram as **"Likely to be Carcinogenic to Humans"** based on tumors in two species and two sexes: a treatment-related increase in thyroid follicular cell adenomas in high dose male mice and liver tumors in female rats at the high dose, with incidences exceeding that of the laboratory's historical controls. There is no mutagenic concern for fluopyram. The available data do not support the proposed modes of action for the thyroid or liver tumors.

The CARC recommended the use of a linear low dose extrapolation model applied to the animal data (Q1*) for quantitative estimation of human risk. The unit risk, Q_1^* $(mg/kg/day)^{-1}$, of Fluopyram based upon female rat liver combined adenoma and carcinoma tumor rates is 1.55×10^{-2} in human equivalents.

I. INTRODUCTION

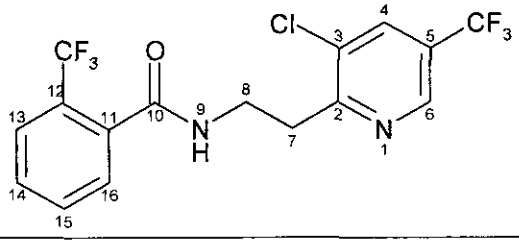
On July 8, 2009, the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of Fluopyram.

II. BACKGROUND INFORMATION

Fluopyram is a global review chemical in partnership with Canada and Germany. Germany has the work share lead for review of toxicology data.

Fluopyram is a new broad-spectrum systemic fungicide of the carboxamide group (FRAC Group 7). It acts on cell respiration in the fungus by inhibiting succinate dehydrogenase (mitochondrial respiration Complex II), thus blocking electron transport.

The main use of fluopyram is the selective control of a variety of fungal diseases like powdery mildew species, *Botrytis cinerea,* and *Alternaria solani* on grape vines and tomatoes. It is also to be used on ornamentals and non-residential turf. Its structure and other pertinent information are depicted in the following table.

Chemical structure:	
Empirical formula:	$C_{16}H_{11}ClF_6N_2O$
Common name:	Fluopyram
Company code:	AE C656948
IUPAC name:	N-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]ethyl}-2-(trifluoromethyl)benzamide
CAS name:	Benzamide, N-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)-(9CI)
CAS no.:	658066-35-4
PC Code	080302

III. EVALUATION OF CARCINOGENICITY STUDIES

1. Combined Chronic Toxicity/Carcinogenicity Study with Fluopyram in Wistar Rats

Reference: Chronic Toxicity and Carcinogenicity Study of AE C656948 in the Wistar Rat by Dietary Administration for 24 months. Bayer Crop Science Project Number: SA/04312, M/298339/01/2. Report No. 04312 February 29, 2008. MRID 47372501

A. Experimental Design

In a combined chronic toxicity and carcinogenicity study, groups of 60 male and female Wistar rats were fed a diet containing 0, 30, 150 and 750 ppm (males) and 0, 30, 150 and 1500 ppm (females) fluopyram for 24 months. In males, the top dose level of 750 ppm had to be reduced to 375 ppm from week 85 onwards because of the high mortality in this group. Over the whole study period, these dietary concentrations corresponded to a mean daily intake of 0, 1.20, 6.0 and 29 mg/kg bw in male rats or 1.68, 8.6 and 89 mg/kg bw in females. In addition, satellite groups of 10 animals per sex and dose were subject to interim sacrifice after 1 year.

B. Discussion of Mortality and Tumor Data

Mortality

Male rats showed a statistically significant trend ($p<0.01$) for mortality with increasing doses of Fluopyram, as well as a significant pair-wise comparison ($p<0.05$) of the 750 ppm dose group with the controls. There was no evidence of significant differences in mortality in female rats (Memo, L. Brunsman, June 10, 2009, TXR #0055218).

Tumors

Female rats had statistically significant trends for liver adenomas ($p<0.01$), carcinomas ($p<0.05$) and combined liver adenomas and carcinomas ($p<0.01$). There were significant pair-wise comparisons of the 1500 ppm dose group with the controls for liver adenomas at $p<0.05$ and for combined liver adenomas and carcinomas at $p<0.01$ (Table 1). There were no statistically significant trends or significant pair-wise comparisons of the dosed groups with the controls for the male rats. The statistical analyses of the tumors in the female rats were based upon Fisher's Exact Test for pair-wise comparisons and the Exact Test for (Memo, L. Brunsman, June 10, 2009, TXR #0055218).

Table 1. Liver Tumor Rates[+] and Fisher's Exact Test and Exact Test for Trend Results in Female Rats

	Dose (ppm)			
	0	30	150	1500
Adenomas (%)	2/59 (3)	2/57 (4)	0/56 (0)	9[a]/55 (16)
p =	0.00049**	0.67765	1.00000	0.01978*
Carcinomas (%)	0/59 (0)	0/57 (0)	2/56 (4)	3[b]/55 (5)
p =	0.02134*	1.00000	0.23494	0.10910
Combined (%)	2/59 (3)	2/57 (4)	2/56 (4)	11[c]/55 (20)
p =	0.00015**	0.67765	0.67083	0.00536**

+Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.
[a]First adenoma observed at week 75, dose 1500 ppm.
[b]First carcinoma observed at week 97, dose 1500 ppm.
[c]One animal in the 1500 ppm dose group had both an adenoma and a carcinoma.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

When compared to historical control data (uncensored data) from the testing laboratory, the incidence of hepatocellular adenomas in the female high dose group at week 104 (9/55, 16.4%) was outside the range of the historical control group (range, 0 - 5%; average, 1.9%). Similarly, the incidence of hepatocellular carcinomas in the female mid and high dose group at week 104 (2/56 (4%) mid and 3/55, 5%, high) exceeded the range of the historical control group (no carcinomas observed in 10 studies from 2000 - 2006).

C. Non-Neoplastic Lesions in the Liver

Non-neoplastic lesions of the liver are presented in Tables 2a and 2b.

Table 2a: Incidence and severity of microscopic changes in the liver, all animals of the carcinogenicity phase (part 1 of 2)

Sex	**Males**				**Females**			
Dose level fluopyram (ppm)	**0**	**30**	**150**	**750/375**	**0**	**30**	**150**	**1500**
Dose level fluopyram (mg/kg)	**0**	**1.20**	**6.0**	**29.0/15.0**	**0**	**1.7**	**8.6**	**89**
Number of animals examined	**60**	**60**	**60**	**58**	**60**	**60**	**60**	**59**
Centrilobular to panlobular hepatocellular hypertrophy: diffuse								
Minimal	1	1	14	15	0	0	0	6
Slight	0	0	0	15	0	0	0	21
Moderate	0	0	0	0	0	0	0	21
Total	1	1	14**	30**	0	0	0	48**
Focus(i) of hepatocellular alteration: clear: focal/multifocal								
Minimal	8	6	6	11	0	3	3	8
Slight	2	2	1	5	1	1	1	3
Total	10	8	7	16	1	4	4	11**
Focus(i) of hepatocellular alteration: eosinophilic: focal/multifocal								
Minimal	12	20	18	15	23	17	14	25
Slight	4	3	10	12	6	7	15	13
Moderate	0	1	2	1	0	2	1	7
Marked	0	0	1	0	0	0	0	3
Total	16	24	31*	28**	29	26	30	48**
Hepatocellular vacuolation: focal/multifocal								
Minimal	10	4	13	7	5	10	7	13
Slight	0	2	3	0	1	4	2	9
Moderate	0	1	0	0	0	0	0	0
Total	10	7	16	7	6	14**	9	22**

*: $p \leq 0.05$, **: $p \leq 0.01$

Table 2b: Incidence and severity of microscopic changes in the liver, all animals of the carcinogenicity phase (part 2 of 2)

Sex	**Males**				**Females**			
Dose level of fluopyram (ppm)	**0**	**30**	**150**	**750/375**	**0**	**30**	**150**	**1500**
Dose level fluopyram (mg/kg)	**0**	**1.20**	**6.0**	**29.0/15.0**	**0**	**1.7**	**8.6**	**89**
Number of animals examined	**60**	**60**	**60**	**58**	**60**	**60**	**60**	**59**
Increased number of mitoses								
Present	0	0	0	0	6	1	5	33
Total	0	0	0	0	6	1	5	33**
Multinucleated hepatocytes with anisocaryosis								
Present	1	0	1	0	4	2	6	38
Total	1	0	1	0	4	2	6	38**
Hepatocellular single cell necrosis: focal/multifocal								
Minimal	2	0	1	1	0	3	1	25
Slight	0	0	1	0	0	1	0	12
Total	2	0	2	1	0	4	1	37**
Hepatocellular brown pigment(s): focal/multifocal								

Sex	**Males**				**Females**			
Dose level of fluopyram (ppm)	**0**	**30**	**150**	**750/375**	**0**	**30**	**150**	**1500**
Dose level fluopyram (mg/kg)	**0**	**1.20**	**6.0**	**29.0/15.0**	**0**	**1.7**	**8.6**	**89**
Number of animals examined	**60**	**60**	**60**	**58**	**60**	**60**	**60**	**59**
Minimal	0	0	0	0	1	1	2	22
Slight	0	0	0	0	0	0	0	2
Total	0	0	0	0	1	1	2	24**
Accumulation of brown pigments in Kupffer cells: focal/multifocal								
Minimal	6	2	9	5	8	7	9	27
Slight	1	0	1	2	4	3	2	4
Moderate	0	0	0	0	0	0	0	1
Total	7	2	10	7	12	10	11	32**
Hepatocellular macrovacuolation: centrilobular to midzonal: diffuse								
Minimal	0	0	0	9	0	0	0	5
Slight	0	0	2	1	0	0	0	4
Moderate	0	0	0	0	0	0	0	2
Total	0	0	2	10**	0	0	0	11**
Extramedullary hematopoiesis: multifocal								
Minimal	16	7	10	15	17	24	21	30
Slight	1	3	1	1	2	1	3	3
Moderate	1	0	0	0	0	0	0	0
Total	18	10	11	16	19	25	24	33**

**: $p \leq 0.01$

D. Adequacy of the Dosing for Assessment of Carcinogenicity

The doses tested were considered to be adequate and not excessive to assess carcinogenic potential in both sexes.

In female rats, the highest dose tested of 1500 ppm was considered to be adequate. This was based on a 20% decrease in mean body weights and increased liver weights (mean: +39%, absolute: +56%), which were dose-related and correlated well with findings such as prominent lobulation and hypertrophy. Other effects that occurred at the high dose included thyroid follicular cell hypertrophy and colloid alteration, chronic progressive nephropathy and kidney tubular dilatation, and retinal atrophy and lenticular degeneration of the eye.

In males, the top dose level of 750 ppm, an excessive dose, had to be reduced to 375 ppm from week 85 onwards because of the high mortality in this group. At the termination of the study, mortality among high-dose males was 50% of control values. The dose of 375 ppm, which is roughly one-half the excessive dose of 750 ppm, however, was considered to be adequate. This was based on decreased mean body weight, increased liver weight, liver hypertrophy, follicular cell hypertrophy and colloid alteration, chronic progressive nephropathy and tubular dilatation and tubular hypertrophy (males) all observed at the high dose.

2. Carcinogenicity Study in C57BL/6J Mice

Reference: Carcinogenicity Study of AE C656948 [fluopyram] in the C57BL/6J Mouse By Dietary Administration for 18 months. Bayer CropScience.Project Number: SA/05094. Report No.05094. December 14, 2007 MRID 47372450

A. Experimental Design

Groups of 60 male and female C57BL/6J mice were fed diets containing 0, 30, 150 or 750 ppm of fluopyram (corresponding to a mean compound intake of 0, 4.2, 20.9 and 105 mg/kg bw/day in males and 0, 5.3, 26.8 and 129 mg/kg bw/day in females, respectively) for up to 78 weeks. After 52 weeks, 10 males and 10 females from each group which had been allocated to the chronic phase of the study were killed and necropsied. The remaining 50 animals per sex and group (allocated to the carcinogenicity phase) continued treatment until scheduled sacrifice after at least 78 weeks of treatment.

B. Discussion of Mortality and Tumor Data

Mortality

There was no evidence of significant differences in mortality with increasing doses of Fluopyram in male or female mice (Memo, L. Brunsman, June 10, 2009, TXR # 0055218).

Tumors

Male mice had a statistically significant trend at $p<0.01$ and a significant pair-wise comparison of the 750 ppm dose group with the controls at $p<0.05$ for thyroid follicular cell adenomas (Table 3). There were no statistically significant trends or significant pair-wise comparisons of the dosed groups with the controls for the female mice. The statistical analyses of the tumors in the male and female mice were based upon Fisher's Exact Test for pair-wise comparisons and the Exact Test for trend (Memo, L. Brunsman, June 10, 2009, TXR # 0055218).

Table 3. Thyroid Follicular Cell Tumor Rates[+] and Fisher's Exact Test and Exact Test for Trend Results in Male Mice

	Dose (ppm)			
	0	30	150	750
Adenomas[#] (%)	1/49 (2)	1/47 (2)	3[a]/48 (6)	7/48 (15)
p =	0.00357**	0.74211	0.30076	0.02758*

+Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.
#No carcinomas were observed.
[a]First adenoma observed at week 79, dose 150 ppm.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

When compared to historical control data (uncensored data) from the testing laboratory, the incidence of thyroid follicular cell adenomas in the male high dose group at week 78 (7/48, 14.6%) was outside the range of the historical control group (range, 0 - 2%; average, 0.4%).

C. Non-Neoplastic Lesions

The non-neoplastic lesions observed in liver and thyroid of both sexes of mice are presented in Tables 4 and 5.

Table 4: Incidence and severity of microscopic changes in the liver, all animals, carcinogenicity phase

Sex	**Males**				**Females**			
Dose level of fluopyram (ppm)	**0**	**30**	**150**	**750**	**0**	**30**	**150**	**750**
Dose level of fluopyram (mg/kg)	**0**	**4.20**	**20.9**	**105**	**0**	**5.30**	**26.8**	**129**
Number of animals	**49**	**49**	**49**	**50**	**48**	**50**	**50**	**50**
Eosinophilic focus(i) of altered hepatocytes: focal/multifocal								
Minimal	1	0	0	0	0	1	2	0
Slight	0	0	0	2	0	0	0	2
Moderate	0	0	0	0	0	0	0	2
Marked	0	0	0	0	0	0	0	1
Total	1	0	0	2	0	1	2	5*
Centrilobular to panlobular hepatocellular hypertrophy: diffuse								
Minimal	0	0	16	3	0	0	18	24
Slight	0	0	22	11	0	0	0	2
Moderate	0	0	0	36	0	0	0	0
Total	0	0	38**	50**	0	0	18**	26**
Number of animals	49	49	49	50	48	50	50	50
Hepatocellular cholestasis: focal/multifocal								

Sex	**Males**				**Females**			
Dose level of fluopyram (ppm)	**0**	**30**	**150**	**750**	**0**	**30**	**150**	**750**
Dose level of fluopyram (mg/kg)	**0**	**4.20**	**20.9**	**105**	**0**	**5.30**	**26.8**	**129**
Number of animals	**49**	**49**	**49**	**50**	**48**	**50**	**50**	**50**
Minimal	0	0	2	29	0	0	0	0
Slight	0	0	0	2	0	0	0	0
Total	0	0	2	31**	0	0	0	0
Hepatocellular single cell degeneration/necrosis: focal/multifocal								
Minimal	1	2	7	28	1	1	0	1
Slight	0	0	0	12	0	0	0	0
Moderate	0	0	0	0	0	0	0	1
Total	1	2	7*	40**	1	1	0	2
Interstitial mixed cell infiltrate: focal/multifocal								
Minimal	18	15	19	39	8	8	10	8
Slight	0	1	0	1	2	3	2	0
Total	18	16	19	40**	10	11	12	8
Eosinophilic inclusion bodies: focal/multifocal								
Minimal	2	3	5	18	0	0	0	0
Slight	0	0	0	1	0	0	0	0
Total	2	3	5	19**	0	0	0	0
Multinucleated hepatocytes: focal/multifocal								
Minimal	3	1	3	25	1	0	0	0
Slight	0	0	1	2	0	0	0	0
Total	3	1	4	27**	1	0	0	0
Hepatocellular vacuolation: mainly centrilobular: diffuse								
Minimal	21	15	13	0	3	4	1	0
Slight	6	19	11	2	2	5	0	1
Moderate	0	4	3	1	0	1	0	0
Total	27	38*	27	3**	5	10	1	1

*: $p \leq 0.05$, **: $p \leq 0.01$

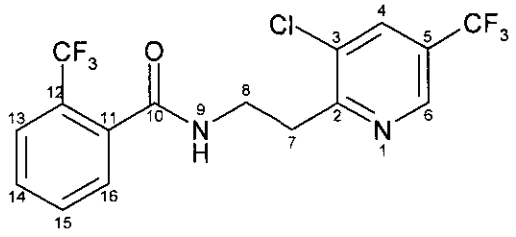
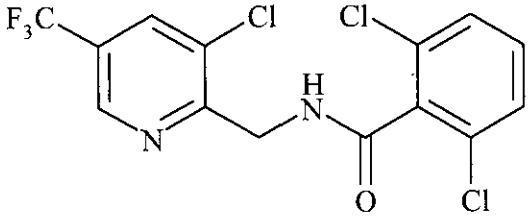
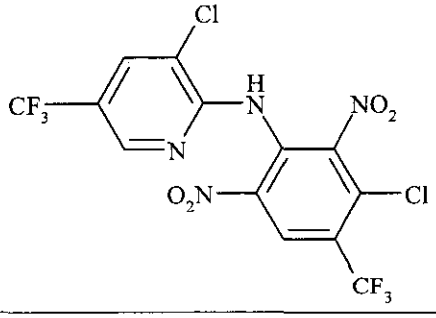
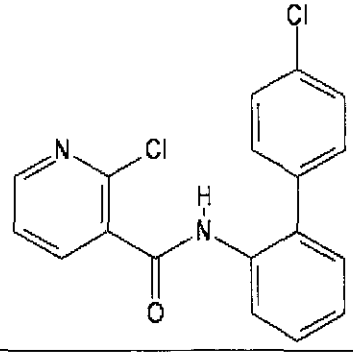
Table 5: Incidence and severity of microscopic changes in the thyroid gland, all animals, carcinogenicity phase

Sex	**Males**				**Females**			
Dose level offluopyram (ppm)	**0**	**30**	**150**	**750**	**0**	**30**	**150**	**750**
Dose level of fluopyram (mg/kg)	**0**	**4.20**	**20.9**	**105**	**0**	**5.30**	**26.8**	**129**
Number of animals	50	50	50	50	48	50	50	50
Follicular cell hyperplasia: focal/multifocal								
Minimal	0	2	10	18	11	4	10	10
Slight	3	2	5	6	5	2	5	12
Moderate	1	2	2	5	1	2	3	6
Marked	0	0	3	3	0	0	1	4
Severe	0	0	1	0	0	0	0	1
Total	4	6	21**	32**	17	8*	19	33**

**: $p \leq 0.01$

3. Structure-Activity Relationship

Fluopicolide, fluazinam and boscalid are considered possible analogs for Fluopyram because they have a benzamide sub-structure and/or a pyridine sub-structure, and share a common use as fungicides. Among the three analogs, fluopicolide is the closest based on chemical structural similarity. The SAR, however, does not inform the mode of action for fluopyram.

Chemical Name	Structure
Fluopyram Benzamide, N-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)-(9CI) CAS Registry No.: 658066-35-4	
Fluopicolide (AE C638206 or 2,6-dichloro-*N*-[[3-chloro-5-(trifluoromethyl)-2-pyridinyl]methyl]benzamide) CAS Registry No.: 239110-15-7	
Fluazinam; IKF-1216 3-Chloro-*N*-[3-chloro-2,6-dinitro-4-(trifluoromethyl)phenyl]-5- (trifluoromethyl)-2-pyridinamine Caswell#: 959 CAS Registry No.: 79622-59-6	
Boscalid BAS 510; 3-pyridinecarboxamide, 2-chloro-N-(4'chloro[1,1'biphenyl]-2-yl) CAS No. 188425-85-6	

In accordance with the EPA's Final Guidelines for Carcinogen Risk Assessment (March 2005), the CARC classified **Fluopicolide** as **"Not Likely to be Carcinogenic to Humans"** based on

convincing evidence that a non-genotoxic, mitogenic mode of action for liver tumors was established in the mouse and that the carcinogenic effects were not likely at doses that do not cause perturbations of the liver.

In accordance with the EPA's Final Guidelines for Carcinogen Risk Assessment (March 2005), **Fluazinam** was classified as a **"suggestive evidence of carcinogenicity to humans"** based on 1) statistically significant increases in hepatocellular adenomas, carcinomas and combined adenomas/carcinomas in male CD1 mice (MRID 42208405); 2) statistically increased incidence of follicular cell adenocarcinomas and combined follicular cell adenomas/ adenocarcinomas in Sprague-Dawley rats (MRID 42248620); equivocal evidence of increased incidence of hepatocellular adenomas, carcinomas or combined adenomas/carcinomas for the male mice (MRID 44807222) Fluazinam is negative in mutagenicity tests.

In accordance with the EPA's Draft Guidelines for Carcinogen Risk Assessment (1996), **Boscalid** was classified as **"suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential"** based on increased incidence of follicular cell adenomas in male (MRID 45404827) and female (MRID 45414828) rats. No evidence of carcinogenicity was found in mice (MRID 45404901). Boscalid is negative in mutagenicity tests.

4. <u>Subchronic and Chronic Toxicity</u>

a) Subchronic Toxicity

Rat (MRID 47372516)

In a 28-day oral toxicity dose-range finding study in rats (MRID 47372516), groups of Wistar rats (5/sex/dose) were administered fluopyram (98.6% purity) in the diet at concentrations of 0, 50, 400 and 3200 ppm (0, 4.0, 31.0 and 254 mg/kg/day in males and 4.6, 36.1 and 263 mg/kg/day in females) for 28 days.

The results showed that at the fluopyram did not produce mortality or clinical signs of toxicity. There were no changes in terminal body weights at any dose in either sex. Increases in platelet counts and prothrombin times were reported in 3200 ppm males. Total cholesterol and triglyceride levels were in increased in 3200 ppm males and females. Liver weights were increased in the 400 and 3200 ppm dose groups in both sexes compared with controls, the effect being slightly more pronounced in females. At 3200 ppm, the increase was more than 50 % compared to controls. This increased weight was associated with enlarged and dark livers at macroscopic examination and with minimal to moderate centrilobular hepatocellular hypertrophy in most animals in both sexes. Similar effects were observed at 400 ppm, however with lower magnitude, incidence and severity. In addition to the liver effects, the thyroid gland weights (absolute and relative) were higher in males at 3200 ppm and 400 ppm. In association, hypertrophy of the follicular cells was observed in 3/5 males. Thyroid gland weight was not

affected in females although 2/5 animals showed a minimal follicular cell hypertrophy.

In addition, a dose-related slight increase in total cytochrome P-450, BROD and PROD activities was observed in both sexes at 3200 and 400 ppm. No significant effects were observed at 50 ppm.

For establishing the NOAEL and LOAEL for this study, the results of the 90-day oral toxicity study should be considered along with those of this study. In this study minimal effects were seen at 400 ppm. However, in the 90-day oral toxicity study, no effects were seen at 200 ppm. At 1000 ppm, similar toxic effects were found as those seen in the 3200 ppm of the 28 day dose- range finding study. Therefore the NOAEL for the 28-day dose-range finding study lies between 400 ppm and 200 ppm (equivalent to 31 to 12.5 mg/kg/day), while the LOAEL is 400 ppm (31 mg/kg/day).

Rat (MRID 47372441)

In a subchronic oral toxicity study (MRID 47372441) fluopyram was administered in the diet for at least 90 days to 10 animals/sex/dose group at 0, 50, 200, 1000, and 3200 ppm (equating approximately to 0, 3.06, 12.5, 60.5 and 204 mg/kg/day in males and 0, 3.63, 14.6, 70.1, and 230 mg/kg/day in females). An additional 10 males and 10 females fed either 0 or 3200 ppm of test diet for at least 90 days were maintained for a minimum of 28 days to examine the reversibility of any effects seen. Following exposure at the prescribed dose level, animals were sacrificed and subjected to toxicological examination.

There were no treatment-related clinical signs in either sex throughout the study. Food consumption rates were essentially similar to controls in all dose groups. At 3200 ppm mean overall body weight gain was reduced by 9% in males ($p \leq 0.05$) and 17% in females ($p \leq 0.01$). The effect on mean body weight was still observed after 4 weeks of recovery in both sexes, as the magnitude of the decrease was similar to that observed at the end of the treatment phase. At 1000 ppm, mean body weight parameters were unaffected by the treatment in either sex, with the exception of a slight decrease of 15% (not statistically significant) in mean body weight gain per day in females during the first week of treatment, compared to controls.

Higher mean prothrombin time was noted at 3200 ppm in males only (+74 %, $p \leq 0.01$), when compared to the control values. Slightly lower mean hemoglobin concentrations were noted at 3200 ppm in both sexes and at 1000 ppm in males only. These variations were associated with lower mean hematocrit in males and lower mean corpuscular volume and mean corpuscular hemoglobin in females at 3200 ppm. Additionally, higher mean platelet (+24 %, $p \leq 0.01$) and reticulocyte (+50 % for absolute count and +42 % for percentage, $p \leq 0.01$) counts were noted at 3200 ppm in females. There was no evidence of treatment-related changes at 1000 ppm in females or at 200 and 50 ppm in either sex. Hematological changes were reversed at the end of the recovery period, with the exception of mean hemoglobin concentrations, which remained

slightly lower than controls.

Several clinical chemistry variations were statistically significant at 1000 ppm or 3200 ppm, and included increased total cholesterol (≥45%), decreased total bilirubin (≥-30%), increased glutamyltransferase (+625%) and triglycerides (+102%). After one month of recovery in the high dose group, there was a general tendency towards reversibility. Nevertheless, statistically significant differences were still noted in mean total cholesterol, globulin concentrations and albumin/globulin ratio in females (+19 %, +14 % and -14 % respectively, $p \leq 0.05$), compared to the control values. The incidence and severity of cellular casts in urine were increased in all groups of fluopyram treated males in a dose-related manner, and correlated with hyaline droplet nephropathy observed at histopathology examinations. However, these effects were largely reversible.

At Week 3, TSH levels were elevated in both sexes at 3200 ppm (+63 %, $p \leq 0.05$ and +71 %, $p \leq 0.01$, respectively), together with an increase in mean T3 and T4 levels in females (+24 %, $p \leq 0.05$ and +54 %, $p \leq 0.01$, respectively), whereas at Week 13, only an increase in mean TSH and T3 levels was noted in males (+88 and +40 %, respectively, $p \leq 0.01$). At 1000 ppm, mean TSH level was increased by 54 % (not statistically significant) and T4 level was significantly increased by 43 % in males on Week 13. A dose dependent increase of T3, T4 and TSH was observed in both sexes at most time points and for most parameters at all dose levels when compared to controls, however, without being significant or of relevant magnitude at the lower two dose levels of 200 and 50 ppm. These changes were reversed in the recovery phase.

Mean body weights were not statistically changed by fluopyram administration. Mean absolute and relative liver weights were statistically increased in males and females at 1000 ppm and 3200 ppm (+ 20 to +25%, and +53% to +74%, respectively). Mean absolute and relative kidney weights were statistically increased predominantly in males (+25% - +34%) at 1000 ppm and 3200 ppm. Thyroid weights were also statistically higher (+10% to +22%) in males and females at 3200 ppm. These various increases were only partially reversed after the one-month recovery period.

Microscopic effects of treatment with fluopyram were seen in the liver, kidney, thyroid gland and lung. In the liver, minimal to moderate centrilobular hepatocellular hypertrophy was observed with a dose-related increase in incidence and severity at 3200 and 1000 ppm in both sexes and minimal centrilobular hepatocellular hypertrophy was observed at 200 ppm in males. In addition, minimal to moderate periportal to midzonal hepatocellular macrovacuolation was observed in females at 3200 and 1000 ppm. In the kidney, hyaline droplet nephropathy (characterized by basophilic tubules, hyaline droplets in proximal tubules and granular casts in the medulla) and hyaline casts were higher at 3200 and 1000 ppm in males, in comparison with controls. Hyaline droplet nephropathy was also slightly higher at 200 ppm in males. In the thyroid gland, a higher incidence of minimal to slight diffuse hypertrophy of follicular cells was seen at 3200 and 1000 ppm in both sexes compared to controls and internal historical control data. After the

recovery period, liver and thyroid gland were comparable between the high dose and control groups, indicating that the changes noted after 90 days of treatment were reversible in these organs. In the kidney in the high dose groups, basophilic tubules, hyaline droplets in proximal tubules, granular casts in the medulla and hyaline casts were persistent in the kidney of some males.

In this study in rats the lowest dose level of 50 ppm represented the No Observed Effect Level (NOEL) in males and females (equating to 3.06 / 3.63 mg/kg body weight/day). At the next higher dose level of 200 ppm (12.5 / 14.6 mg/kg bw/d), however, treatment-related effects were confined to a rather adaptive liver weight increase due to hepatocellular hypertrophy that was associated with non-significant changes in clinical chemistry parameters. Because these findings were not considered adverse, the NOAEL for both sexes was set at 200 ppm (12.5 / 14.6 mg/kg bw/day), and the LOAEL is 1000 ppm (60.5 / 70.1 mg/kg bw/day) based on organ weight changes, and clinical chemistry and histopathological findings in liver, thyroid and kidneys.

Mouse (MRID 47372517)

In a 28-day oral toxicity dose-range finding study in mice (MRID 47372517), groups of C57BL/6J mice (5/sex/dose) were administered fluopyram (98.4% purity) in the diet at concentrations of 0, 150, 1000 and 5000 ppm (0, 24.7, 162, and 747mgkg/day in males and 0, 31.1, 197, 954 mg/kg/day in females) for 28 days. The mg/kg dose equivalent for the high dose males (747 mg/kg/day) was calculated for weeks 1-3 only due to mortality or early sacrifice in this group.

At 5000 ppm, all males and 3/5 females were sacrificed prior to scheduled sacrifice. All unscheduled sacrificed animals presented the following clinical signs: reduced body weights, decreased motor activity, hunched posture, piloerection, wasted appearance and/or coldness to touch in both sexes together with labored respiration, and distended abdomen. The two surviving females at 5000 ppm, had elevated total cholesterol (+118 %) and total protein (+16 %) concentrations, and alanine aminotransferase activities (+384 %). Mean absolute and relative liver weights were higher at 5000 ppm in females and at 1000 and 150 ppm in both sexes. These changes were found to be dose-related. Gross pathology showed enlarged and dark livers males and females, the Thymus size was reduced in 4/5 males and 1/3 females, and distended abdomen was noted in 3/5 males. Red liquid was observed in the thoracic cavity in all males. Histopathology indicated. Histopathology demonstrated hypertrophy of the hepatocytes in males and females and hepatocellular focal necrosis was also noted in surviving females.

At 1000 ppm, there was a slight decrease in the daily mean body weight gain during study Week 2 in males. No treatment-related effect on mean body weight and mean body weight change parameters was noted at 1000 ppm in females or 150 ppm in either sex. No effect on mean food consumption was noted in animals surviving to terminal sacrifice. Enlarged liver was seen in all males and in 4/5 females. Hepatocellular hypertrophy was seen in all 1000 ppm males

and 2/5 females, and focal necrosis was found in 2/5 females.

At 150 ppm, there was a slight increase in liver weights, and liver hypertrophy was seen in all males and 3/6 females.

In consideration of NOAEL and LOAEL, the results show that hepatocellular hypertrophy at 150 ppm (24.7 mg/kg/day) (LDT), but currently liver hypertrophy is considered to be an adaptive effect. However, more pronounced liver effects (hypertrophy and focal necrosis) are seen at 1000 ppm and above. NOAEL for this study can be established at 150 ppm (24.7 mg/kg/day). LOAEL is 1000 ppm (162 mg/kg/day) based on increased incidence of liver hypertrophy and focal necrosis in the liver.

Mouse (MRID 47372442)

In a sub-chronic oral toxicity study (MRID 47372442), fluopyram was administered in the diet for at least 90 days to C57BL/6J mice at the following doses – 0, 30, 150 and 1000 ppm (equating approximately to 0, 5.4, 26.6 and 188 mg/kg/day in males and 0, 6.8, 32.0 and 216 mg/kg/day in females). A negative control group received plain diet. Animals were then sacrifice and subjected to toxicological examination.

There were no treatment-related clinical signs of toxicity observed in animals at any dose level. No treatment-related mortality occurred in the test animals at any dose level. Body weight and food consumption were not affected by treatment.

Blood analysis: At 1000 ppm, mean alanine aminotransferase activity was higher in males and females (+205 and +109 % after removal of an outlier in the female control values, respectively, $p \leq 0.01$), compared to the control groups. In addition in males, mean alkaline phosphatase activity was higher (+21 %, $p \leq 0.01$), mean albumin and mean total cholesterol concentrations were lower (-12 and -40 %, respectively, $p \leq 0.01$) and a tendency towards higher values was noted in aspartate aminotransferase activity (+46 %, $p \leq 0.05$). In females, a tendency towards lower mean albumin concentration was also noted (-9 %, $p \leq 0.01$). At 150 treatment-related changes consisted in a lower mean total cholesterol concentration in both sexes (-41 and -30 %, respectively, $p \leq 0.01$) and lower total protein and albumin concentrations in males (-6 % and – 7 %, $p \leq 0.01$), associated with lower total bilirubin in males and females (- 43 %, significant only in males) compared to the control group. At 30 ppm the only observed finding was a significant decrease in total cholesterol in males (-30 %, $p \leq 0.01$).

There was no relevant change in terminal body weight in either sex. Mean absolute and/or relative liver weights were statistically significantly higher at 1000 ppm (+36% to +45%) and 150 ppm (+14% to +28%) in both sexes, with a dose-related effect. At 30 ppm, mean absolute liver weight and relative liver to body weight ratio were statistically significantly higher (+11% to +13%) in females only, but this change was considered not to be toxicologically relevant since

it was not associated with any histological change. Mean absolute and relative adrenal gland weights were increased by between 87 % and 92 % at 1000 ppm in males compared to controls, the difference being statistically significant for mean adrenal gland to body weight ratio only ($p \leq 0.05$). At 1000 ppm, enlarged livers were observed in 8/10 males and 9/10 females, and dark livers were observed in 5/10 males and 10/10 females.

Effects of treatment with fluopyram were seen in the liver and adrenal gland in both sexes. In the liver, there was a minimal to moderate hypertrophy of centrilobular hepatocytes in both sexes at 1000 and 150 ppm. This finding correlated with the enlarged livers observed at necropsy in animals given 1000 ppm and the statistically significant increase in liver weight seen in animals given 1000 or 150 ppm. Also observed was a greater incidence of minimal or slight focal necrosis in both sexes at 1000 ppm, when compared to controls. Minimal or slight focal necrosis was present in 3/10 males and 6/10 females given 1000 ppm, compared to one female in the control group. In the adrenal glands at 1000 ppm, there was a lower incidence of cortical steroid pigment in males and a greater incidence of minimal to slight cortical vacuolation in females, compared to controls. The change noted in males at 1000 ppm was considered to be slight compared to the magnitude of increase in adrenal gland weights seen in this sex. No treatment-related changes were observed in the adrenal glands at 150 or 30 ppm in either sex. In the fore-stomach of males given 1000 ppm, there was a slightly greater incidence of focal epithelial hyperplasia than in controls. However, as these changes in males were only focal and isolated, they were considered not to be treatment-related. In females, the incidence and severity of this finding were comparable between controls and treated animals.

The NOAEL for this study is 150 ppm (equating to 26.6 and 32.0 mg/kg bw/day in males and females respectively). The LOAEL is 1000 ppm (approximately 118 or 216 mg/kg bw/day for males and females, respectively) based on liver effects (hypertrophy, necrosis, changes in related clinical chemistry parameters).

b) Chronic Toxicity

Rat (MRID 47372501)

In a combined chronic toxicity and carcinogenicity study (MRID 47372501), groups of 60 male and female Wistar rats were fed a diet containing 0, 30, 150 and 750 ppm (male animals) and 0, 30, 150 and 1500 ppm (females) fluopyram for 24 months. In males, the top dose level of 750 ppm had to be reduced to 375 ppm from week 85 onwards because of the high mortality in this group. Over the whole study period, these dietary concentrations corresponded to a mean daily intake of 0, 1.20, 6.0 and 29 mg/kg bw in male rats or 1.68, 8.6 and 89 mg/kg bw in females. In addition, satellite groups of 10 animals per sex and dose were subject to interim sacrifice after 1 year.

Overall, there was a statistically significant increase in mortality in males at 750/375 ppm during

the study and after 24 months although no clear cause for these premature deaths could be established. At the highest dose levels of 750/375 in males or 1500 ppm in females, respectively, mean body weights were significantly reduced in both sexes at various times throughout the study. The main target organs were the liver, kidneys and the thyroid gland, and also the eyes.

Liver toxicity became apparent by an increase in organ weight at the two upper dose levels in male rats and in the highest dose group in females. This was sometimes accompanied by gross pathological findings such as nodules/masses which correlated histologically with neoplastic changes.

Clinical chemistry findings suggesting hepatotoxicity were minor in nature and confined to the respective top dose level in both sexes. They comprised occasionally higher mean triglyceride concentrations and slightly lower mean glucose concentrations in the females. Activity of alkaline phosphatase was reduced in both sexes throughout the study but achieved statistical significance only occasionally. Histological changes included a higher incidence of altered hepatocytes (eosinophilic foci) and hepatocellular brown pigments, focal or multifocal hepatocellular vacuolation, increased number of mitoses, centrilobular to panlobular hypertrophy and hepatocellular single cell necrosis with females being much more affected. Again, this might be due to the higher dose that the female rats were fed. At the mid dose level of 150 ppm, however, histopathological lesions (hypertrophy) were confined to male rats and, thus, were in line with the increase in organ weight that was noted in the same sex only.

In the kidneys, marked degenerative changes such as chronic progressive nephropathy or focal/multifocal (medullar or cortical) tubular dilatation, together with an increased incidence of tubular golden/brown pigments (mainly in females) and collecting ducts hyperplasia, were observed at the high dose level. In addition, a higher incidence of hyaline droplets and of renal cysts was noted in male rats. At the mid dose level of 150 ppm, male rats still displayed a higher frequency of tubular hypertrophy or dilatation. During the first year of treatment, but not thereafter, urinalysis revealed higher incidences of abnormal color of urine (orange to red) in females and a higher incidence and severity of cellular casts in males. This latter finding was also confirmed in male rats receiving the intermediate dose.

Effects of fluopyram on the thyroid gland were demonstrated by an increased organ weight at the highest dose level in both sexes. This finding was associated with histopathological changes (follicular cell hyperplasia and/or hypertrophy, colloid alteration/depletion) that were of less severity or frequency but yet to be noted at the mid dose level, too.

In addition, the eyes were affected by long-term treatment. Ophthalmologic examination revealed abnormal color of the retinal fundus in females after 12 months. At the 24-month examination, this condition was observed in females and males, together with small retinal vessels. In addition, hyperreflectivity in the retina was noted in females and corneal opacity, edema of the cornea and nuclear opacity in males. These effects were more severe at the top dose level and less

pronounced but still present after 2 years in the male and female groups receiving the intermediate dose. Histologically, bilateral retinal atrophy was noted at the highest dose level, together with a higher incidence of lens degeneration.

A tendency towards lower erythrocyte parameters (hemoglobin concentration, mean corpuscular volume, hematocrit and/or mean corpuscular hemoglobin) was observed in the female high dose group throughout the study confirming evidence from short-term studies that the red blood cells might be an additional target. The same tendency was observed in high dose males at most time points, however, statistical significance was not achieved. The assumption of an effect on the blood was further substantiated by a more frequent occurrence of extramedullary hematopoiesis in the livers of high dose females. In contrast to the subchronic study, prothrombin time was this time shorter in the female high dose group with the difference being significant at months 6 and 12.

Therefore, the NOAEL was 30 ppm (1.2 and 1.7 mg/kg bw/day in males and females, respectively). The LOAEL of 150 ppm (6.0 and 8.6 mg/kg/day, males and females), is based on nephropathy in the kidney, follicular cell hypertrophy in the thyroid, and increased liver weight with gross pathological and histopathological findings.

Mouse (MRID 47372450)

In a mouse carcinogenicity study (MRID 47372450), groups of 60 male and female C57BL/6J mice were fed a diet containing 0, 30, 150 or 750 ppm of fluopyram (corresponding to a mean compound intake of 0, 4.2, 20.9 and 105 mg/kg bw/day in males and 0, 5.3, 26.8 and 129 mg/kg bw/day in females, respectively) for up to 78 weeks. After 52 weeks, 10 males and 10 females from each group which had been allocated to the chronic phase of the study were killed and necropsied. The remaining 50 animals per sex and group (allocated to the carcinogenicity phase) continued treatment until scheduled sacrifice after at least 78 weeks of treatment.

There were no unscheduled deaths or clinical signs occurring during the study that could be attributed to treatment. The survival rate was not different among the control and dose groups. Body weight gain was decreased only in high and mid dose males and only during the second trimester of the study (weeks 26 to 54). Afterwards, some compensatory growth was observed resulting in a mean final body weight that was similar to the control group value.

A slightly higher mean platelet count was determined in top dose males but no other changes in hematological parameters were seen.

Pathological examination revealed that the liver, the thyroid gland and the kidneys were the main target organs in mice.

Mean absolute and relative liver weights were markedly increased in high and mid dose males

and females at interim sacrifice as well as at study termination. The increment exhibited a clear dose response. At these two upper dose levels, gross necropsy findings such as dark and enlarged livers were corroborated by an increase in non-neoplastic histopathological lesions such as centrilobular to panlobular hypertrophy, hepatocellular cholestasis, single cell degeneration/necrosis or eosinophilic foci. A few of these non-neoplastic effects on the liver were observed only in males pointing to a higher vulnerability of this sex with regard to hepatotoxicity.

Toxic effects on the thyroid were noted at the top dose level in both males and females and in the mid dose male group. The main non-neoplastic finding, *i.e.*, follicular cell hyperplasia became apparent in male mice at interim sacrifice already. In line with that, the only neoplastic change in this study consisted of a higher incidence of follicular cell adenoma in high dose males (7/50) as compared to the control group (1/50). This difference was statistically significant ($p \leq 0.05$).

Mean absolute and relative kidney weights were decreased at the 750 ppm dose level in both sexes. In addition, a higher incidence and/or severity of bilateral cortical basophilic tubules, hyaline casts(s) and interstitial mononuclear cell infiltrates, glomerular congestion/ hemorrhage(s), and more pronounced amyloid deposition (mainly in the glomerular interstitium) was noted at this dose but only in females.

Thus, the No Observed Adverse Effect Level (NOAEL) in this study was 30 ppm in males (equivalent to 4.2 mg/kg/day) and females (equivalent to 5.3 mg/kg/day). The LOAEL was 150 ppm (equivalent to 20.9 mg/kg bw/day in males and 26.8 mg/kg bw/day in females) based on hepatocellular hypertrophy in the liver in both sexes and follicular cell hyperplasia in the thyroid gland in males.

5. Mode of Action

1. Mode of Action for Thyroid Follicular Cell Tumors in Male Mice

The registrant, Bayer CropScience, submitted a proposed mode of action for the thyroid follicular cell tumors seen in male mice using the International Programme on Chemical Safety (IPCS) framework (MRID 47753406). Their postulated MOA for fluopyram-induced thyroid follicular cell tumors in mice involved the perturbation of homeostasis of the pituitary–thyroid axis by an extra thyroidal mechanism that is not the result of genotoxicity. The key events proposed included: 1) increases in liver enzyme activity, similar in pattern to the phenobarbital-induced enzyme activity pattern, 2) alterations in thyroid and pituitary hormone levels, 3) increase in thyroid growth, and 4) lesion progression in the thyroid from hyperplasia to neoplasia. Thyroid follicular cell adenomas in male mice were associated with chronic exposure to 105 mg/kg/day fluopyram. In addition to the subchronic and chronic studies, the registrant submitted MOA studies to further elucidate the MOA for fluopyram exposing mice for short durations (3, 4 and 14 days) to doses of 300 mg/kg/day (MRID No. 47567128, 47372519, 47690101). A summary of

the results of this MOA analysis is provided in the table below.

Mode of Action-- Thyroid Tumors in Mice			
Key Events	**Animal Evidence**	***Dose Concordance***	***Temporal Association***
Induction of Liver Enzymes	3-day day mechanistic study at 2000 ppm, 308 mg/kg/day [MRID 47567128] 14-day mechanistic study at 2000 ppm or 314 mg/kg/day [MRID 47372519] Increased Total CYT P450, PROD, EROD and BROD at 2000 ppm	**No.** Studies were performed at dose levels higher than the tumorigenic dose of 105 mg/kg/day.	Yes
Hormone Changes (Decreased T3, T4, Increased TSH)	3-day day mechanistic study at 2000 ppm, 308 mg/kg/day [MRID 47567128] 4-day mechanistic study at 200 ppm [MRID 47690101] 14-day mechanistic study at 2000 ppm or 314 mg/kg/day [MRID 47372519] No decreases in T3 observed. Some decreases in T4 observed. Increase in T4 clearance over 24 hrs. Increase in TSH.	**No.** Studies were performed at dose levels higher than the tumorigenic dose.	Yes
Increases in cell growth (Increased thyroid weight, Increased thyroid hypertrophy and hyperplasia)	Mouse carcinogenicity Study 0, 30, 150, 750 ppm (0, 4.2/5.3, 20.9/26.8, 105/129 mg/kg/day, Males/Females) Thyroid hyperplasia observed at 20.9 and 105 mg/kg/day at 1 year and 18 months. Not seen at 90 days [MRID: 47372450] No increase in thyroid weight and no thyroid hypertrophy observed in subchronic or chronic mouse studies.	**Yes**, some evidence of thyroid hyperplasia observed at and below the tumorigenic dose of 750 ppm (105 mg/kg/day)	Yes
Thyroid follicular cell tumors	Mouse carcinogenicity Study [MRID: 47372450]	Tumors observed at 750 ppm (105 mg/kg/day)	--

The CARC evaluated the submitted MOA and concluded that there is insufficient data to support the proposed MOA for fluopyram induced thyroid follicular cell tumors. The major deficiency in the analysis was the lack of dose-response concordance with key events and tumors. The MOA studies were performed at doses that were above the tumorigenic dose. There is no data for several key events at or below the tumorigenic dose, which is required as experimental support for the MOA. While temporal association was established, the missing dose concordance data precludes an acceptable MOA. In addition,

while a comparison to Phenobarbital MOA is informative, the MOA for fluopyram must stand on its own.

2. Mode of Action for Liver Tumors in Female Rats

The registrant, Bayer CropScience, submitted a proposed mode of action for the liver tumors in female rats using the IPCS framework (MRID 47753406). They postulated a mitogenic MOA for fluopyram-induced liver tumors. It involved the induction of liver enzymes, including cytochrome P450s (CYP450) isozymes, consequent liver cell proliferation and hypertrophy, and altered hepatic foci, ultimately progressing to neoplasia. The specific MOA for fluopyram was considered by the registrant to be similar to the phenobarbital MOA for liver tumors in rats. Liver tumors in female rats were associated with chronic exposure to 89 mg/kg/day (1500 ppm) fluopyram. In addition to the subchronic and chronic studies, the registrant submitted a MOA study to further elucidate the MOA for fluopyram exposing rats for short durations (7 days) to a dose of 190 mg/kg/day (3000 ppm) (MRID 47372520). A summary of the results of the MOA analysis is provided in the table below.

Mode of Action—Mitogenesis Liver Tumors in Rats			
Key Events	**Animal Evidence**	**Dose Concordance**	**Temporal Association**
Nuclear Receptor *Activation*	No	--	--
Increased Induction of CYP Phase I Enzymes	7-day mechanistic study at 3000 ppm, 193 mg/kg/day [MRID 47372520]	No. Study was performed at dose levels higher than the tumorigenic dose of 1500 ppm, 89 mg/kg/day.	Yes Measured at 7 days and 28 days
	28-day subchronic study with liver enzyme induction at 50, 400 and 3200 ppm, 4.6, 36.1 and 263 mg/kg/day [MRID 47372516]	Yes. BROD and PROD elevated at 400 ppm, below tumorigenic dose	
Increased Liver Weight	7-day mechanistic study at 3200 ppm, 193 mg/kg/day [MRID 47372520]	No. Studies were performed at dose levels higher than the tumorigenic dose of 1500 ppm, 89 mg/kg/day.	Yes Measured at 7 days, 28 days, 90 days and 2 years
	28-day subchronic study at 50, 400 and 3200 ppm, 4.6, 36.1 and 263 mg/kg/day [MRID 47372516]	No. Liver weight increase seen only at 3200 ppm, higher than tumorigenic dose.	
	90-day subchronic. at 50, 200,1000, 3200 ppm, 3.63,14.6, 70.1, 230 mg/kg/day [MRID 47372441]	Yes. Liver weight changes seen at 1000 and 3200 ppm	

Mode of Action—Mitogenesis Liver Tumors in Rats			
Key Events	**Animal Evidence**	**Dose Concordance**	**Temporal Association**
Increased Liver Hypertrophy and Hyperplasia	28 day subchronic study at 3200 ppm [MRID 47372516] 90 subchronic study at 3200 ppm [MRID 47372441] Chronic/Oncogenicity study at 1500 ppm [MRID 47372501]	No. Hypertrophy seen only at 3200 ppm, higher than tumorigenic dose. Yes. Hypertrophy seen at 1000 and 3200 ppm Yes. Hypertrophy seen at 1500 ppm, the tumorigenic dose.	Yes Hypertrophy observed at 7 days, 28 days, 90 days and 2 years
Increased Cell Proliferation	7-day mechanistic study [MRID 47372520]	No. Studies were performed at dose levels higher than the tumorigenic dose.	No time-course data on cell proliferation. Tested only at 7 days
Inhibition of Apoptosis	No	--	--
Liver Tumors	Chronic/Oncogenicity study [MRID 47372501]	Tumors observed at 1500 ppm (89 mg/kg/day)	

The CARC evaluated the submitted MOA and concluded that there was insufficient data to support the proposed MOA for fluopyram induced liver tumors. The major deficiency in the analysis was the lack of dose-response concordance with key events and tumors. The MOA studies were performed at doses above the tumorigenic dose. There is no MOA data at or below the tumorigenic dose, which is required as experimental support for the MOA. In addition, there is no time course data on cell proliferation. Ideally, a study of several weeks duration is needed with early time points (minimum of 2 time points of 90 days or less) to demonstrate that cell proliferation is not sustained. The data submitted was not sufficient and tested only 1 dose and 1 time point. While temporal association was established for several key events, the missing dose concordance data and adequate cell proliferation data preclude an acceptable MOA. In addition, while a comparison to Phenobarbital MOA is informative, the MOA for fluopyram must stand on its own.

V. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

The Committee considered the following for a weight-of-evidence determination of the carcinogenic potential of fluopyram:

1. Carcinogenicity

Rat

• Administration of fluopyram resulted in the induction of liver tumors in female Wistar rats. There were statistically significant trends for liver adenomas ($p<0.01$), carcinomas ($p<0.05$) and combined liver adenomas and carcinomas ($p<0.01$). There were significant pair-wise comparisons of the 1500 ppm dose group with the controls for liver adenomas at $p<0.05$ and for combined liver adenomas and carcinomas at $p<0.01$. When compared to historical control data (uncensored data) from the testing laboratory, the incidence of hepatocellular adenomas in the female high dose group (9/55, 16%) was outside the range of the historical control group (range, 0 - 5%; average, 1.9%). Similarly, the incidence of hepatocellular carcinomas in the female mid (2/56, 4%) and high dose groups (3/55, 5%), while not statistically significant by pair-wise comparison, exceeded the range of the historical control group (no carcinomas observed in 10 studies from 2000 - 2006) and was considered to be biologically relevant. There were no statistically significant trends or significant pair-wise comparisons of the dosed groups with the controls for the male rats. **The CARC considered the liver tumors in female Wistar rats to be treatment-related.**

• *Adequacy of Dosing*: The doses tested were considered to be adequate and not excessive to assess carcinogenic potential in both sexes. **In female rats**, the highest dose tested of 1500 ppm was considered to be adequate. This was based on a 20% decrease in mean body weights and increased liver weights (mean: +39%, absolute: +56%), which were dose-related and correlated well with findings such as prominent lobulation and hypertrophy. Other effects that occurred at the high dose included thyroid follicular cell hypertrophy and colloid alteration, chronic progressive nephropathy and kidney tubular dilatation, and retinal atrophy and lenticular degeneration of the eye. **In male rats**, the top dose level of 750 ppm, an excessive dose, had to be reduced to 375 ppm from week 85 onwards because of the high mortality in this group. At the termination of the study, mortality among high-dose males was 50% of control values. The dose of 375 ppm, which is roughly one-half the excessive dose of 750 ppm, however, was considered to be adequate. This was based on decreased mean body weight, increased liver weight, liver hypertrophy, follicular cell hypertrophy and colloid alteration, chronic progressive nephropathy and tubular dilatation and tubular hypertrophy (males) all observed at the high dose.

Mouse

• Administration of fluopyram resulted in the induction of thyroid follicular cell tumors in male C57BL/6J mice. Male mice had a statistically significant trend at $p<0.01$ and a significant pair-wise comparison of the 750 ppm dose group with the controls at $p<0.05$ for thyroid follicular cell adenomas. There were no statistically significant trends or significant pair-wise comparisons of the dosed groups with the controls for the female mice. When compared to historical control data (uncensored data) from the testing laboratory, the incidence of thyroid follicular cell adenomas in the male high dose group (7/48, 15%) was outside the range of the historical control group (range, 0 - 2%; average, 0.4%). **The CARC considered the thyroid follicular cell adenomas in male C57BL/6J mice to be treatment-related.**

• *Adequacy of Dosing*: The high dose of 750 ppm was considered to be adequate, and not excessive, to assess carcinogenic potential in both male and female mice, based on the following: 1) Increased absolute and relative liver weights in males and females at the high- and mid-dose levels. These changes were dose-related, and correlated well with macroscopic findings (dark/enlarged livers) and non-neoplastic histopathological lesions (eosinophilic foci [females], hepatocellular hypertrophy [both sexes], cholestasis [males], single cell degeneration/necrosis [males]); 2) Increased incidence of thyroid follicular cell hyperplasia in both sexes at the high dose; and 3) Decreased absolute and relative kidney weights in both sexes at the high dose level, along with increased incidence and/or severity of kidney lesions (bilateral cortical basophilic tubules, hyaline casts and interstitial mononuclear cell infiltrates, glomerular congestion/ hemorrhage, and amyloid deposition) in females.

2. Mutagenicity

There is no concern for mutagenicity.

3. Structure-Activity Relationship

The SAR data do not inform the mode of action.

4. Mode of Action

The CARC concluded that insufficient data were provided to support definitive modes of action for the induction of liver tumors in female rats or thyroid follicular cell tumors in male mice. Deficiencies included a lack of dose-response concordance with key events and tumors.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA's *Final Guidelines for Carcinogen Risk Assessment* (March, 2005), the CARC classified fluopyram as **"Likely to be Carcinogenic to Humans"** based on tumors in two species and two sexes: a treatment-related increase in thyroid follicular cell adenomas in high dose male mice and liver tumors in female rats at the high dose, with incidences exceeding that of the laboratory's historical controls. There is no mutagenic concern for fluopyram. The available data do not support the proposed modes of action for the thyroid or liver tumors.

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The CARC recommended the use of a linear low dose extrapolation model applied to the animal data (Q_1*) for quantitative estimation of human risk.[1] The unit risk, Q_1^* $(mg/kg/day)^{-1}$, of Fluopyram based upon female rat liver combined adenoma and carcinoma tumor rates is 1.55×10^{-2} *in human equivalents (Memo. L. Brunsman, 11/17/09, TXR No. 0055280).*

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1 At the July 8, 2009 meeting, the CARC recommended a classification of "Likely" with no quantification. Following this meeting, as per the recommendation of the CARC chair, a revote was requested to be consistent with the cancer guidelines. Subsequently, the CARC recommended a "Likely" classification with quantification.

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